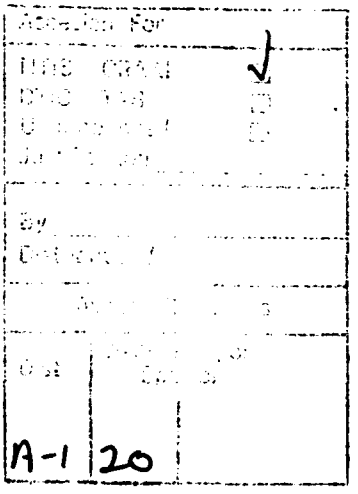
INTERNATIONAL SYMPOSIUM ON TICK-BORNE ARBOVIRUSES

(Excluding group B)

Publishing House of the Slovak Akademy of Sciences
Bratislava

SECTION I

CLASSIFICATION OF ARBOVIRUSES TRANSMITTED BY TICKS



SEROLOGICAL AND PHYSICO-CHEMICAL CONSIDERATIONS

J. Casals

Arboviruses whose vectors are, or are suspected of being, ticks can be divided into two sets; those that belong in group B and those that do not. In this Symposium only the latter are considered.

The tick-borne viruses not of group B (non-GBTB) viruses constitute an increasingly larger collection which consists at present of about 40 distinct serotypes. None is, thus far, antigenically related to arboviruses outside the collection. As with other arboviruses, the evidence that they are tick-borne is fairly conclusive in few instances; in most cases it is assumed on the basis that, with few exceptions, ticks are the only arthropods from which they have been isolated in nature.

In this part of the lecture are included three sections: 1. Serological considerations. 2. Physico-chemical considerations. 3. Studies on serotypes of timely interest.

1. Serological considerations

Methods. The process of antigenic characterization of non-GBTB viruses does not differ from that of the other arboviruses.

Hyperimmune sera or ascitic fluids are prepared in mice and antigens for in vitro tests are obtained from infected organs of newborn mice, generally from the brain but on occasion from the liver. Only about one-fourth of these viruses have yielded hemagglutinating (HA) antigens for goose erythrocytes. These antigens have usually had low titers, of an order of magnitude between 1:40 - 1:160, high avidity, i.e., were not

easily inhibited by immune sera and poor patterns of sedimentation with slippage and roughness. These poor qualities as well as the relatively low number of positives are responsible for the fact that the hemagglutination-inhibition (HI) test has not, thus far, contributed greatly to the study of these agents.

The antigens are, on the other hand, effectively used in the complement-fixation (CF) test; all strains have yielded positive preparations with titers between 1:32 and 1:2048, mostly between 1:64 and 1:512.

The neutralization (N) test, either by inoculation of the serum-virus mixtures to mice or to cells in culture tubes, has also been extensively used in the work reported here; and, more recently, agar gel diffusion and precipitation has been applied to these pathogens.

Once the reagents are available, the process of characterization of a presumed non-GBTB virus consists in determining its possible relationship (identical, related but distinct, unrelated) first with any arbovirus outside the set and second with other viruses of the set. The first part is done mainly by HI, by screening an immune serum for the problem virus against all, or as many as seem necessary and sufficient, viruses that produce HA antigens; in the absence of the latter the screening is completed by CF test.

The comparison of a candidate strain with the established non-GBTB viruses has been done mainly by CF test; if a relationship is thereby detected it is further analyzed by other serological methods, particularly N test.

The currently established serotypes are listed in Table 1. While studies with all the agents listed, except NSD, Ponteves and Karamoja have been carried out in this laboratory, it is not to be assumed that their characterization is solely the result of our work; a large number of these agents have been submitted to the „WHO, World Reference Center for Arboviruses, YARU", with various degrees of valid antigenic information.

It is furthermore to be noted that there may exist divergent opinions concerning the interpretation of the results on which is based the definition of the listed names as distinct serotypes. Thus Quaranfil and Johnston Atoll viruses, being closely related, could be considered as subtypes of a virus. Conversely, Rudnick et al. (1967) consider some strains of Wad Medani sufficiently distinct to deserve recognition as separate viruses.

Certain antigenic relationships among viruses in Table 1 have been established by CF test with the result that, thus far, 22 have been

classified in 7 antigenic groups, shown in Table 2; the remaining viruses remain ungrouped.

The experimentally observed relationships on which these groups, are based vary in degree. In the Ganjam, Kaisodi and Uukuniemi groups, for example, the members are distantly related; while in the Quaranfil group, as stated earlier, the 2 members are closely related, both by CF and N tests. The relationships in the, at present largest of the groups, Kemerovo group,are discussed in detail later.

2. Physico-chemical considerations

Classification of viruses in a general or universal system, such as that proposed by the ICNV (1965)is based exclusively on properties of the virion. Regardless of the outcome of that proposal it is understandable that, for many reasons, the essential properties of the virion should be known, particularly those on which proposed classifications are based, namely, type of nucleic acid, presence or absence of velope, symmetry, and size.

Insofar as they have been studied, the non-GBTB viruses have been found to contain RNA and to be inactivated by ethyl ether or sodium deoxycholate, thus presumably having a lipid-containing envelope. Much less is known concerning their symmetry. Reingold et al. (1964) described three types of isometric particles associated with Kemerovo virus, which they considered as having icosahedral symmetry; the larger of these particles being presumably the enveloped virion had an estimated size, by electron microscopy, between 60 and 100 mμ. Saikku and von Bonsdorff (1968) reported that Uukuniemi virus under the electron microscope appeared as spheres 90-100 mμ in diameter, with details on the surface that looked like capsomeres; later (personal communication) they observed long, helical structures emerging from

*A number of these viruses have not as yet been described in the literature; their names or laboratory designations are used here with the kind permission and cooperation of the following persons responsible for their isolation:

C-5581, Dr. R.L. Doherty; DGK and Hazara, Drs. C.L. Wisseman, Jr., and F. Begum; Dugbe, Dr. O.R. Causey; Farallon, Mono Lake and Huacho, Dr. H.N. Johnson; Grand Arbaud and Ponteves, Dr. Cl. Hannoun; Lone Star, Dr. R.H. Kokernot; Mal P6-1361, Dr. N.J. Marchette; Mutucare, Dr. K.M. Johnson; Pak Argas 461 and Pak T487, Dr. H.C. Barnett; and Qalyub, Dr. R.M. Taylor.

the virion, but did not conclude that this virus was a myxovirus due to the fact the helix was loose and easily ruptured. Murphy et al. (1968) reported that Colorado tick fever (CTF) virions appeared as spherical particles, 80 mμ in diameter, with suggestive cubic symmetry; they described similar characteristics for Chenuda virus.

Filtration of a number of these viruses through graded Milipore membranes (Casals, 1968) gave estimated diameters as follows: Chenuda and Kemerovo, about 60 to 80 mμ; Bhanja, CTF, Ganjam, Hughes, Kaisodi, Mutucare, Quaranfil, Silverwater, Thogoto, Tribeč, and Wad Medani, between 78-80 and 140-150 mμ; Nyamanini gave on repeated occasions results that would indicate a size between 140-150 and 300 mμ. Subsequently we have determined that Congo virus was also in the class between 70-80 and 140-150 mμ.

3. Studies on serotypes of timely interest

Kemerovo group. The antigenic relationships among the members of this group, determined by CF using mouse hyperimmune sera, are illustrated by the combined results shown in Table 3.

It is apparent from these results that there are in the group two complexes and one unattached virus. The Kemerovo-Lipovník-Tribeč close relationship has previously been described by various laboratories; the second complex, Chenuda, Mono Lake and Huacho viruses, has only recently been observed and it is still possible that the last two agents are but sub-types of a virus. Finally, the position of Wad Medani (Eg Ar 492) is one of distant attachment; possibly other strains of this virus, or, as reported above by Rudnick et al. (1967) of other members of the Seletar complex, may help in clarifying the position of this agent in the Kemerovo group, which may indeed consist of three loosely connected complexes.

In Table 4 are given the results of N tests with four viruses; no information is as yet available for the others. More detailed studies by N test using tissue cultures are reported elsewhere in this Symposium (Buckley, 1969); the N tests in mice have failed consistently to detect a relationship between Chenuda virus and the members of the Kemerovo complex.

Uukuniemi group. Investigations on the relationships among members of this group have been so far done with the CF and HI tests;

the latter could be used to some advantage as all members of the group have given agglutinating antigens.

As can be seen in Table 5, there is no difference by CF between strains S 23 and Poteplí, for which reason and while awaiting results of N tests these strains are considered as one virus. The relationship between these two strains and Grand Arbaud (Ar 27), although distant, has been reproduced repeatedly; on the other hand, the connection between Pak Ar 461 and the remaining viruses requires confirmation. An additional virus, Ponteves, isolated by Hannoun (personal communication) was found by him to be related to this group.

The result of an HI test, Table 6, appears to give a sharp definition of the group in that reciprocal and significant cross-reactions were observed; the possibility of extending the use of this test to other non-GBTB viruses employing improved antigens (Ardoin et al., in press) is being explored.

Crimean Hemorrhagic Fever and Congo virus. The etiology and other aspects of CHF is fully covered in another section of this Symposium. For this reason no more will be reported here except to state that strains of a virus isolated from patients with the disease were kindly submitted to YARU by Professor Chumakov; one of the strains, Drosdov, has been found to be closely related to, if not identical with, several strains of Congo virus. The similarity has been noted by CF, N and agar gel precipitation tests (Casals, 1969).

Conclusion. Serological investigations of the increasingly larger set of non-group B tick-borne viruses have resulted in the establishment of definite antigenic groups as well as in the detection of strains of the same agent in widely separated areas of the world.

References

Ardoin P., Clarke D.H. and Hannoun Cl. (1969): The preparation of arbovirus hemagglutinins by sonication and trypsin treatment. Amer. J. Trop. Med. Hyg. 18, 592.

Buckley S.M. (1969): In vitro propagation of tick-borne viruses other than group B arboviruses. International Symposium on Tick-borne Arboviruses (Excluding Group B), Smolenice, 1969.

Casals J. (1968): Filtration of arboviruses through „Millipore" membranes. Nature 217, 648.

Casals J. (1969): Antigenic similarity between the virus causing Crimean hemorrhagic fever and Congo virus. Proc. Soc. exp. Biol. Med. 131, 133.

Committee (1965): Proposals and recommendations of the Provisional Committee for Nomenclature of Viruses (P.C.N.V.). Ann. Institute Pasteur 109,625.

Murphy F.A., Coleman P.H., Harrison A.K., and Gary G. D. Jr. (1968): Colorado tick fever virus: An electron microscopic study. Virology 35, 28.

Reingold V.N., Shestopalova N.M., Grachov V.P., Karpovich L.G. and Chumakov M.P. (1964): Electron microscopic study of the morphology of Kemerovo virus using the negative staining technique. Proc. IIIrd European Regional Conference on Electron Microscopy, Prague 1964, p. 353.

Rudnick A., Marchette N.J. and Garcia R. (1967): Seletar, a new Wad Medani-related arbovirus from Malaysia and Singapore. Proceedings 1st South East Asian Regional Seminar on Tropical Medicine, Bangkok 1967, p. 43.

Saikku P. and von Bonsdorff C.H. (1968): Electron microscopy of the Uukuniemi virus, an ungrouped arbovirus. Virology 34, 804.

Table 1

Tick-borne viruses not of group B

Name	Strain reference, YARU	Name	Strain reference, YARU
Bandia	IPD/A 611	Mal P 6-1361	Mal P6-1361
Bhanja	IG 690	Mono Lake	Cal Ar 861
C-5581	C-5581	Mutucare	MARU 21343
Chenuda	Eg Ar 1152	Nairobi Sheep Disease#	
Colorado tick fever	Condon	Nyamanini	Eg Ar 1304
Congo *	3010	Pak Argas 461	Pak Argas 461
DGK	JD 254	Pak T 487	Pak T 487
Dugbe	Ib Ar 1792	Ponteves#	
Farallon	Cal Ar 846	Huacho	Cal Ar 883
Ganjam	IG 619	Qalyub	Eg Ar 370
Grand Arbaud	Argas 27	Quaranfil	Eg Ar 1095
Hazara	JC 280	Sawgrass	TA 14-64A-1247
Hughes	Dry Tortugas	Seletar	SM-214
Johnston Atoll	LBJ	Silverwater	M 3737
Kaisodi	G 14132	Soldado	Tr 52214
Karamoja#	AMP 6839	Thogoto	2 A
Kemerovo	R-10	Tribeč	Original
Lanjan	TP 94	Uukuniemi	S 23
Lipovník	Lip 91	Wad Medani	Eg Ar 492
Lone Star	TM 1381	Wanowrie	G 700

*Includes Drosdov strain, CHF.
#Not investigated at YARU.

Table 2
Antigenic group of tick-borne viruses not of group B

Group designation	Viruses in Group
Ganjam	Dugbe, Ganjam
Hughes	Farallon, Hughes, Soldado
Kaisodi	Kaisodi, Lanjan, Silverwater
Kemerovo	Chenuda, Kemerovo, Lipovník Mono Lake, Huacho, Tribeč, Wad Medani*
Qalyub	Bandia, Qalyub
Quaranfil	Johnston Atoll, Quaranfil
Uukuniemi	Grand Arbaud, Pak Argas 461 Uukuniemi

*Includes Seletar. Membership subject to confirmation.

Table 3
Kemerovo Group
Complement-fixation test

Antigen	Serum						
	Kem	Lip	Tri	Che	ML	Hu	Wm
Kemerovo	512/128	64/32	64/32	8/8	16/32	0	0
Lipovník	64/64	256/64	128/64	8/8	8/8	0	Traces
Tribeč	64/256	128/256	128/256	8/32	16/128	0	Traces
Chenuda	16/16	32/32	32/32	256/128	64/64	16/8	Traces
Mono Lake	16/16	8/16	32/16	32/16	256/64	64/32	
Huacho	8/8			16/16	128/32	256/32	
Wad Medani	0		8/16				128/64

Reciprocal of serum titer/reciprocal of antigen titer; first dilution, 1:4.

Table 4
Kemerovo Group
Neutralization tests by the intracerebral route in mice
Protection given as log of neutralization index

Serum		Virus			
Virus	Species	Kem	Lip	Tri	Che
Kemerovo	Mouse	5.5	1.7	1.7	0.6
	Rat	3.6	0.6	0.5	-0.1
Lipovník	Mouse	2.2	3.6	2.5	0.5
	Rat	1.9	3.8	2.6	0.0
	Rabbit	0.8	2.4	1.3	-0.1
Tříbeč	Mouse	0.7	1.6	2.5	
	Rat	0.7	2.4	3.3	0.1
	Rabbit	0.7	2.0	4.4	0.1
Chenuda	Rat	0.8	<0.5	<0.3	4.0
	Rabbit	0.1	<0.5	<0.7	3.7

Table 5
Uukuniemi Group
Complement-fixation test

Antigen	Serum			
	S 23	Pot	GA	461
Uukuniemi, S 23	256/128	512/128	8/8	0
Uukuniemi, Potepli	128/256	512/256	8/8	0
Grand Arbaud	16/16	16/16+	256/256	Traces
Pak Argas 461	0	0	8/8	512/512

See Table 3.

Table 6
Uukunieme Group
Hemagglutination-inhibition test

Serum	Antigen, 8 Units						
	Uu	GA	461	487	Lan	Bha	Quar
Uukuniemi	640	40	40	0	0	0	0
Grand Arbaud	160	160	40	0	0	0	0
Pak Argas 461	80	20	160	0	0	0	0
Pak T 487	0	0	0	1280	0	0	0
Lanjan	0	0	0	0	80	0	0
Bhanja	0	0	0	0	0	80	0
Silverwater	0	0	0	0	0	0	0
Quaranfil	0	0	0	0	0	0	80
Johnston Atoll	0	0	0	0	0	0	320

Reciprocal of serum titer; first dilution, 1:10.